NATURE OF GREEN OR OFFCOLOR CONDITION IN PRECOOKED YELLOWFIN TUNA

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United States Department of the Interior, Fred A. Seaton, Secretary
Fish and Wildlife Service

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ABSTRACT

Preliminary results from the analytical use of spectral reflectance in the study of the "green condition in precooked yellowfin tuna are reported. It was found possible to predict tendency greening from laboratory precooking. The preliminary conclusions were that color changes in t are the results of oxidative changes in the hemoglobin and myoglobin pigments of the flesh, and differences in color, including greening, result from differences in the concentration of these p ment derivatives. A relatively higher concentration of methemoglobin or metmyoglobin in raw flesh seems to be indicative of a tendency to greening on precooking.

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The color of foodstuffs is often an rtant factor governing their acceptance or tion by the processor and consumer. An esting example of the influence of color in egard is the "greening" of the flesh of cer- una that becomes evident on precooking to canning. The exploration of the fishery rces of the central Pacific by the Pacific nic Fishery Investigations of the U. S. Fish Vildlife Service, and the use of the longline ch large deep-swimming yellowfin tuna, unnus macropterus (Temminck and gel), have drawn attention to this particu- olor problem because of the relatively percentage of tuna prone to "greening" catch made by this type of gear.

The Japanese have long recognized the em, particulary in their winter catch of re, Germo alalunga (Bonnaterre), in the Pacific and have given some study to its . The conclusions reached as a result of investigations are that greenness is asso- with low oil content, with low weight-to- relationship, and is a physiological con- unrelated to freshness. Some of these igators feel that the wasting effects of ruggles of the fish endeavoring to free elves from the hook in the longline method hing have much to do with the discoloration quently encountered (Miyauchi 1950).

More recently certain Japanese investiga- have reported a correlation between the ing of tuna and the concentration of a flesh nt spectrophotometrically identified by as myoglobin. They also claim to be able edict the occurrence of greening with 85 nt certainty by visual examination of the heat (Matsuzaka and Takahashi MS.).

t is the purpose of this report mmarize the conclusions reached as a of a preliminary investigation on this em conducted at the University of Hawaii 1955-56 under contract No. 14-19-008- with the Pacific Oceanic Fishery Investi- s. We are indebted to Mr. Fred Jermann waiian Tuna Packers, Ltd., Honolulu, for tance in preparing samples in the cannery and for other services that have contributed to the study. Thanks are also due to Dr. Albert L. Tester, Director, and Mr. Garth I. Murphy, Assistant Director of the Pacific Oceanic Fishery Investigations, and to other members of the staff for help and suggestions in this work.

COLOR IN FOODS

The problem of the definition and specification of color is a highly complicated one, the detailed discussion of which would be out of place here. Suffice it to say that physiological as well as physical factors are involved. In other words, the "properties" of the observer must be considered as well as the spectral distribution at the source of color. Many systems (C.I.E., Munsell, U.S.C., for example) have been set up and many instruments devised to facilitate an objective definition of color. It has been recognized, however, the the spectrophotometric curve, the plot of reflected or transmitted intensity versus wave-length, is "the most unambiguous specification of color that can be obtained," and that the spectrophotometer is the basic instrument in the standardization of color (Mackinney and Chichester 1954). Recognition should be made of the limitations of such curves in the evaluation of color as it will appear to the eye since perceived color can be greatly altered by slight variations in spectral curves, and two different curves may give the same color response to the eye. In addition to the dominant wave-length (hue), the factors of whiteness-purity (chroma) and photometric brightness (value) are also to be considered in color specification.

THE COLOR OF NORMAL AND GREEN TUNA

In studying the color problem in tuna flesh it was necessary to establish objectively the normal color of desirable or acceptable meat. Accordingly precooked meat judged to be of good quality was selected and designated as normal. In order to define objectively the color of this meat, it was decided to measure its reflectance over the visible range of

wave-lengths of light (400 to 700 millimicrons) using the Beckman DU spectrophotometer with reflectance attachment. To obtain a diffuse or matt surface, and eliminate factors such as gloss and directional color, the meat was forced through a 16-mesh stainless steel screen and packed heaping full into a 1 1/4-inch aluminum planchet. A glass plate was pressed firmly on to the meat until a uniform and smooth surface was produced. The filled planchet was introduced into the reflectance attachment of the spectrophotometer and measurement was made in comparison with a pure high-fired alumina disk covered by a similar glass plate.

A typical series of such normal spectral reflection curves is given in figure 1. The reproducibility of the individual readings was within an average of five-tenths percent reflectance unit for tuna flesh, although for color stable materials, such as mixtures of powdered colored glasses, no variation was discernible. Reflectance curves measured on replicate samples from the same region of the fish loin gave an average precision of 1.5 percent reflectance units. Reflection from cooked tuna meat is generally high, being highest in the red end. The existence of the Soret absorption at about 410 millimicrons points to heme protein pigments as the source of color. It should be noted that absorption "peaks" lead to decreased reflectance, and so appear as troughs on the percent reflectance curves used in this report.

No attempt was made to fix the specification o this color on Munsell or similar systems sinc comparison, rather than standardization, wa involved in the study. The complicating facto of oxidative browning was present, and cause a general lowering in reflectance with tim after cooking (fig. 1a). At times a heighteni of color (increased reflectance) was noted o frozen storage in metal foil wrapping (fig. 1b but this may have been due to unusual samp variation. Further work to eliminate or contr this additional browning variable is in process

Samples similarly judged by experts to be green were prepared and measured. A typical reflectance curve is shown in figure 2 with a non-browned normal curve shown for compari son. It will be observed that curves for green and normal are somewhat similar save for a general decrease in brightness and the indication of a pigment absorbing in the blue (440-500 millimicrons) wave-lengths for the green meat. It seems anomalous that the perceived effect i an apparent green since less blue-green reflec tion is involved. The effect may be due to the higher proportionate red-to-blue reflectance for normal than for green tuna, since the ratio of reflectance of red (640 millimicrons) to blue (410 millimicrons) was 4.7 for normal and 3.5 for green flesh. The effect can be emphasized by reference to figure 3, which shows the reflectance curve of tuna meat that has been soaked in a green dye solution (malachite green

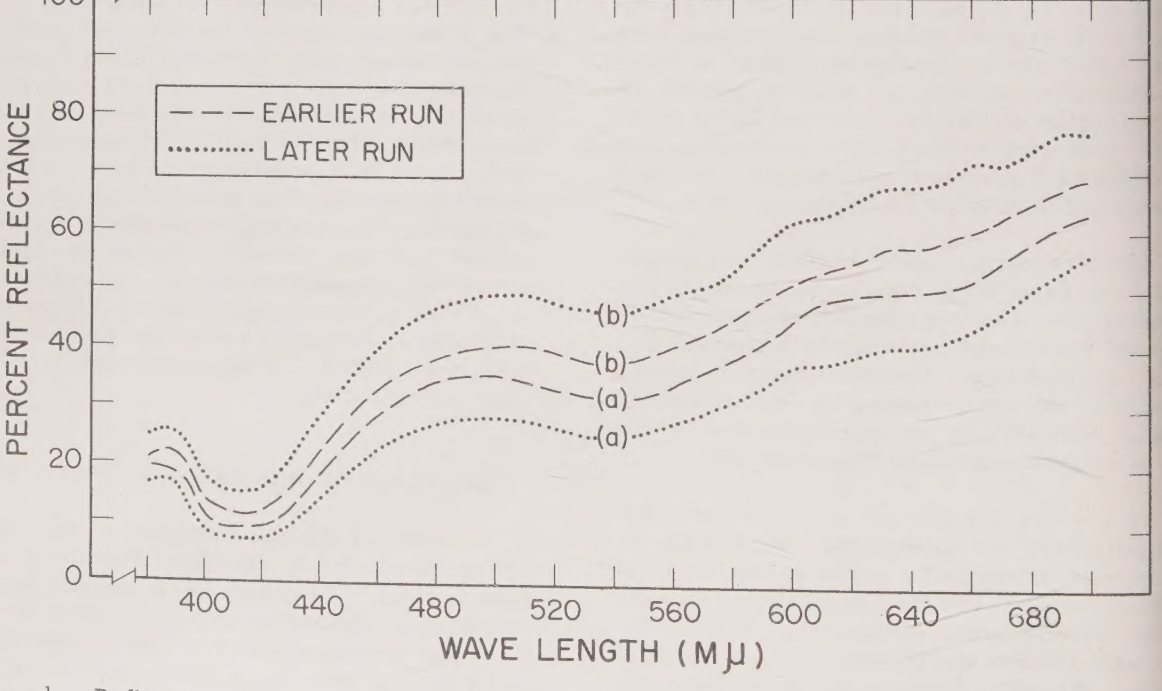

Figure 1.--Reflectance of normal cooked tuna meat: (a) an unwrapped sample in which reflectance decreased with time, (b) a metal foil wrapped sample in which reflectance increased with time.

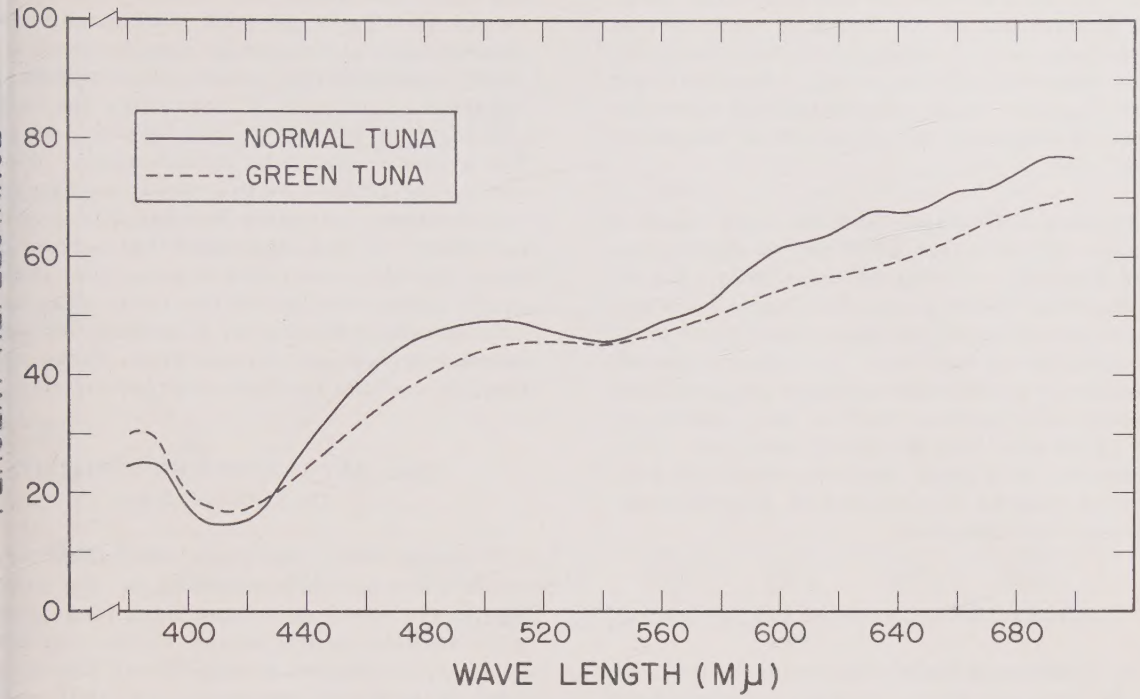

Figure 2.--Comparative reflectance of normal (solid line) and green (dashed line) tuna.

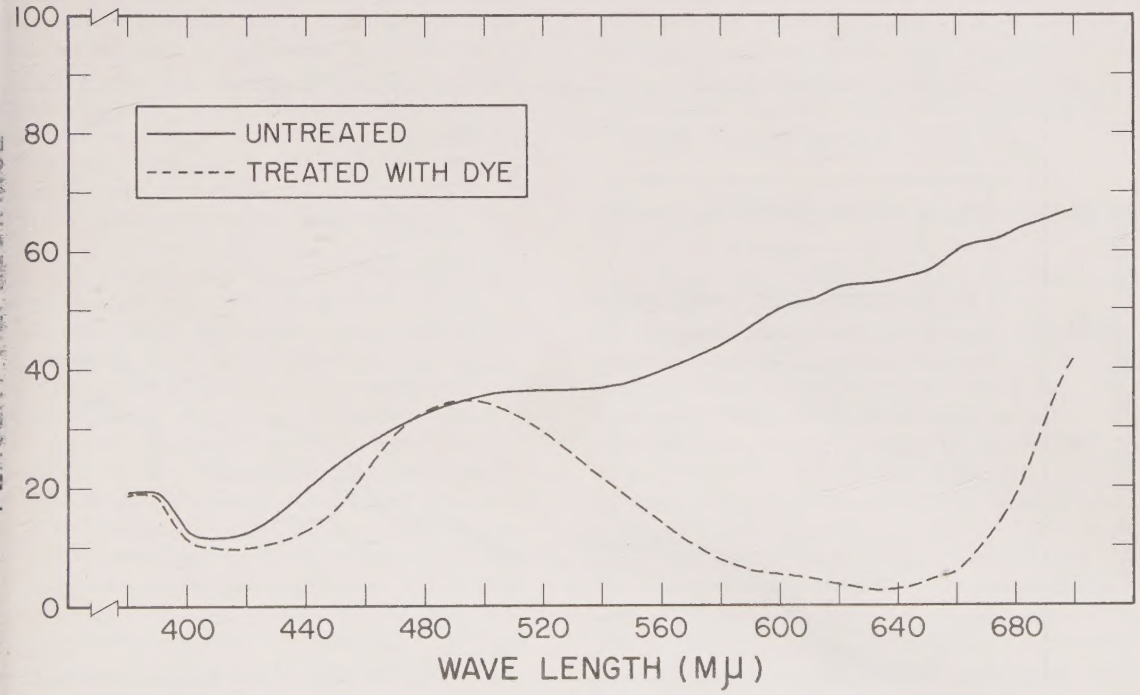

Figure 3.--Reflectance of cooked tuna meat with malachite green dye added and without green dye added.

in comparison with an untreated sample. An increased absorption in the red end of the spectrum is noted due to the pigment, with normal or less than normal reflection in the blue-green region (400-500 millimicrons). Similarly the slightly greater absorption in the red noted for green tuna meat may be the source of the green effect.

Similar reflectance studies were made on raw tuna whose color behavior on cooking was known (fig. 4). It will be noted that, for the few samples tested thus far, there is some correlation between known greenness and the reflectance of the raw flesh. A tendency toward normal color is shown by a higher proportionate reflectance in the red end of the spectrum (light "pinkness") in uncooked samples. The relationship is rather tenuous, therefore further experiments are needed to establish its existence with certainty.

LABORATORY COOKING METHOD

In order to simplify the magnitude of the cannery procedures necessary to produce greening, laboratory cooking was attempted. It was found that the color of 1-inch cubes of meat cooked in a pressure cooker at 102°C. indicated whether or not the fish from which they were taken had any tendency to turn green under commercial methods. A 10-minute ing time was sufficient to show this tende longer periods gave an increased darke (browning?) of the meat. Spectrophotome reflectance curves, which summarize the difference produced by laboratory and com cial methods of cooking can be seen in figu The green produced by the laboratory met was not as intense as that produced by com cial methods, probably because of concomi browning. It was also noted that normal co flesh was less desirable in color than that duced commercially for the same reason. was necessary therefore, in making compari between green and normal flesh, to be cer that the cooking methods corresponded.

THE NATURE OF THE PIGMENT IN TUNA FLESH

From what has been noted thus far, evident that the understanding of the proce that cause greening in tuna flesh must awai understanding of the nature of the pigment volved. It has been pointed out that the e ence of the Soret absorption peak (410 milli crons) in the reflectance curves is indica of the presence of a heme protein, howev this still leaves the question of the exact nat of the heme compound unsettled. It would expected that myoglobin, the hemoglobin fo

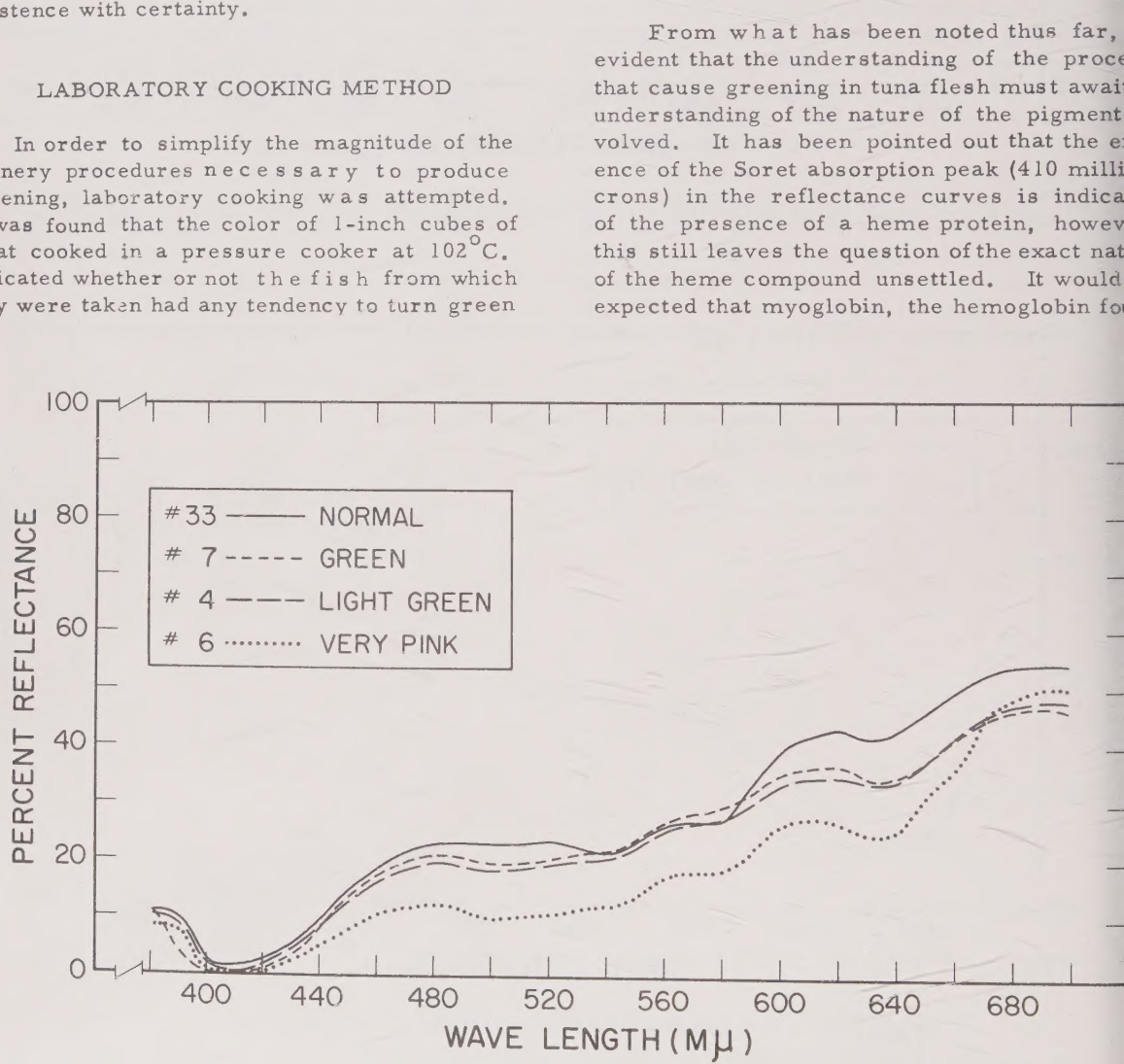

Figure 4.--Reflectance of raw tuna meat of known color behavior on cooking.

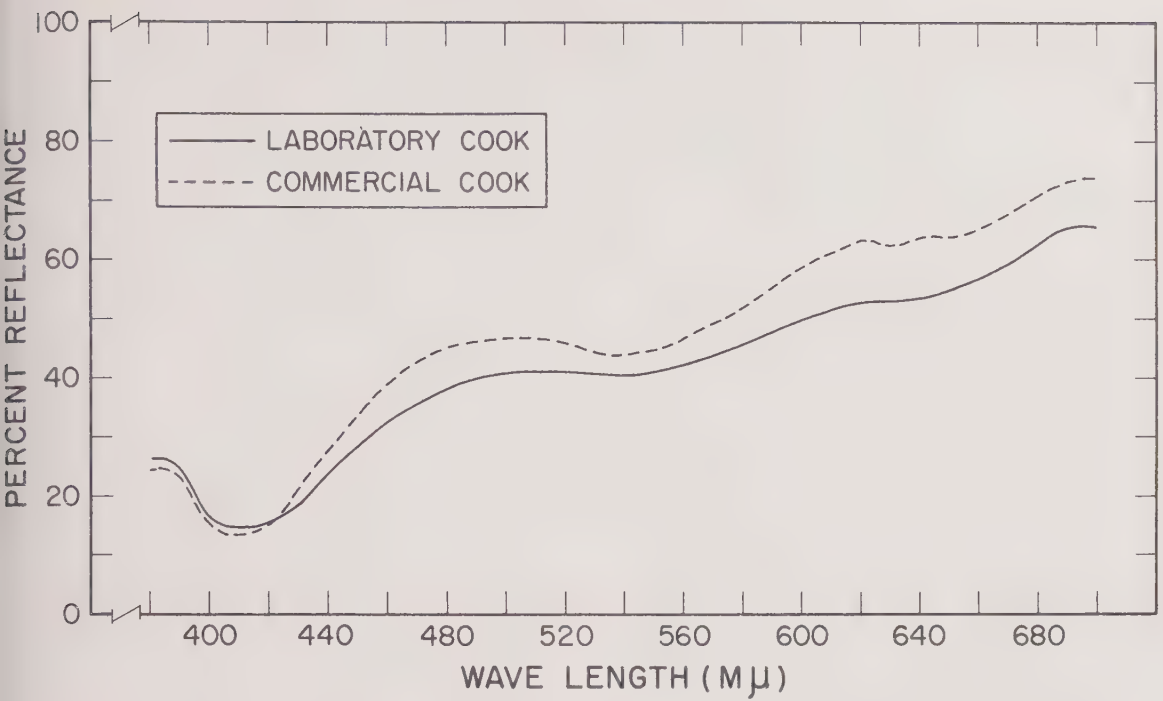

Figure 5.--Reflectance of cooked normal tuna prepared in the laboratory and commercially at Hawaiian Tuna Packers, Ltd.

eat tissue, would be present, and this, in has been reported in albacore by Matsuzaka Takahashi (MS.). Moreover, myoglobin has reported in the red blood meat of fish flesh ther investigators (Mondovi and Antonini). In the species of tuna (yellowfin), under tigation in this study, the evidence seems int to similar pigments or their derivatives involved, despite the fact that earlier es of the properties of the pigment led us spect a peroxidase-like heme protein.

Part of the work on this project involved an pt to extract pigments from tuna flesh. A effort was made to obtain green substances he verdohemochrome type from the green . In the case of raw meat it was found pos- with aqueous or dilute alcoholic solvents tain an extract that contained much of the ent of the meat. The final simplified pro- re settled on was essentially that of Husaini . (1950), and involved blending and extraction a dilute carbonate solution. Other solvents, as alcohol and glacial acetic acid, extracted ents which seemed to be altered products ably by denaturation or cleavage) of the ents present in the flesh. No success was ved in extracting pigments from cooked s.

Close examination of the spectophotometric es of the pigment extracted from raw flesh shows it to be the ferric oxidized form of hemoglobin or myoglobin (methemoglobin or metmyoglobin). This is easily reducible to the ferrous form. Further work is under way through derivative formation to establish whether hemo- or myoglobin is involved, with the greater probability favoring the latter, judging from the findings of the Japanese workers.

CHANGES IN PIGMENT PRODUCING GREENING

In order to elucidiate the mechanism or reason for greening, attempts were made to fractionate the pigmented mixture extracted from green and normal raw tuna flesh. Use was made of ammonium sulfate fractionation and filter paper electrophoresis, both without success to date. Extraction studies seem to show the presence, or the extractability, of a larger amount of methemo- or metmyoglobin from green meat than from normal. Reflectance studies also are in agreement with this since a lesser degree of reflectance seems to be indicated for the average green than for the average unbrowned normal tuna flesh, showing a higher concentration of the absorber or pigment. Certain normal samples do show an abnormal pinkness with high pigment concentration (e.g., No. 6, fig. 4). Further work is under way to separate more positively the factors of browning and greening, and to show the relationship, if any, between the two.

It is interesting to examine the reflectance curves with the possibility of qualitatively and, in a relative way, quantitatively making an estimate of the pigments present. It would be expected that the reflectance curves would resemble the well-known absorption curves for the heme pigments that might be involved, and examination reveals this to be the case.

Raw Meat (fig. 4). Two conditions will be noted, first, a normal type of curve with a variation in over-all position (brightness) due to variation in the amount of pigment present (compare curves for samples No. 6 and No. 33, fig. 4), and second, curves of a different shape (compare curves No. 4 and No. 7 with curve No. 33, fig. 4). Disregarding the Soret peak (about 410 millimicrons), other "peaks" (actually depressions in the curve) can be detected at 540 and 580 millimicrons, indicative of oxyhemoglobin or oxymyoglobin, and at 500 and 630 millimicrons, indicative of methemo- or metmyoglobin. If comparison is made with normal flesh (No. 33), it will be noted that the concentration of the oxy-compound is about the same in green and normal (No. 4 and No. 7, and No. 33). A higher concentration of the methemoglobin or metmyoglobin is indicated by reduced percent reflectance for the green tuna, however. The over-all condition seems to be, then, that similar pigments are present in green and normal raw flesh, with a higher concentration of oxidized methemoglobin or metmyoglobin in the green.

Various experiments were tried in attempt to artificially produce color changes tuna flesh. It was found that soaking raw nor flesh in 0.3 percent hydrogen peroxide soluti imparted greenness after cooking. Also, soal raw incipient green flesh in reducing agents s as ascorbic acid improved the color of the gr meat after cooking. These effects are what w be expected from the normal reactions of her or myoglobin pigments with hydrogen peroxi or with reducing agents.

Cooked Meat (fig. 2). On cooking, the r exhibits similar variations in the reflectar curves as noted for raw flesh (fig. 4). T curves reveal maximum absorption at about and 575 millimicrons, characteristic of denat globin hemichrome. Absorption maxima also noted at about 650 millimicrons and green fish a significant flattening in the regi of 440 to 490 millimicrons, probably also d to absorption. Neither of these latter regio of absorption can readily be connected with mon pigments of the hemo-myoglobin type their derivatives.

By way of a further check on these obser tions, samples of the same cooked meat w reduced with dithionite and the reflectance cu were examined (fig. 6). Save for the abnorm pink sample (No. 6), all samples were foun have about the same reflectance. A pink c was induced in the green meat on reduction

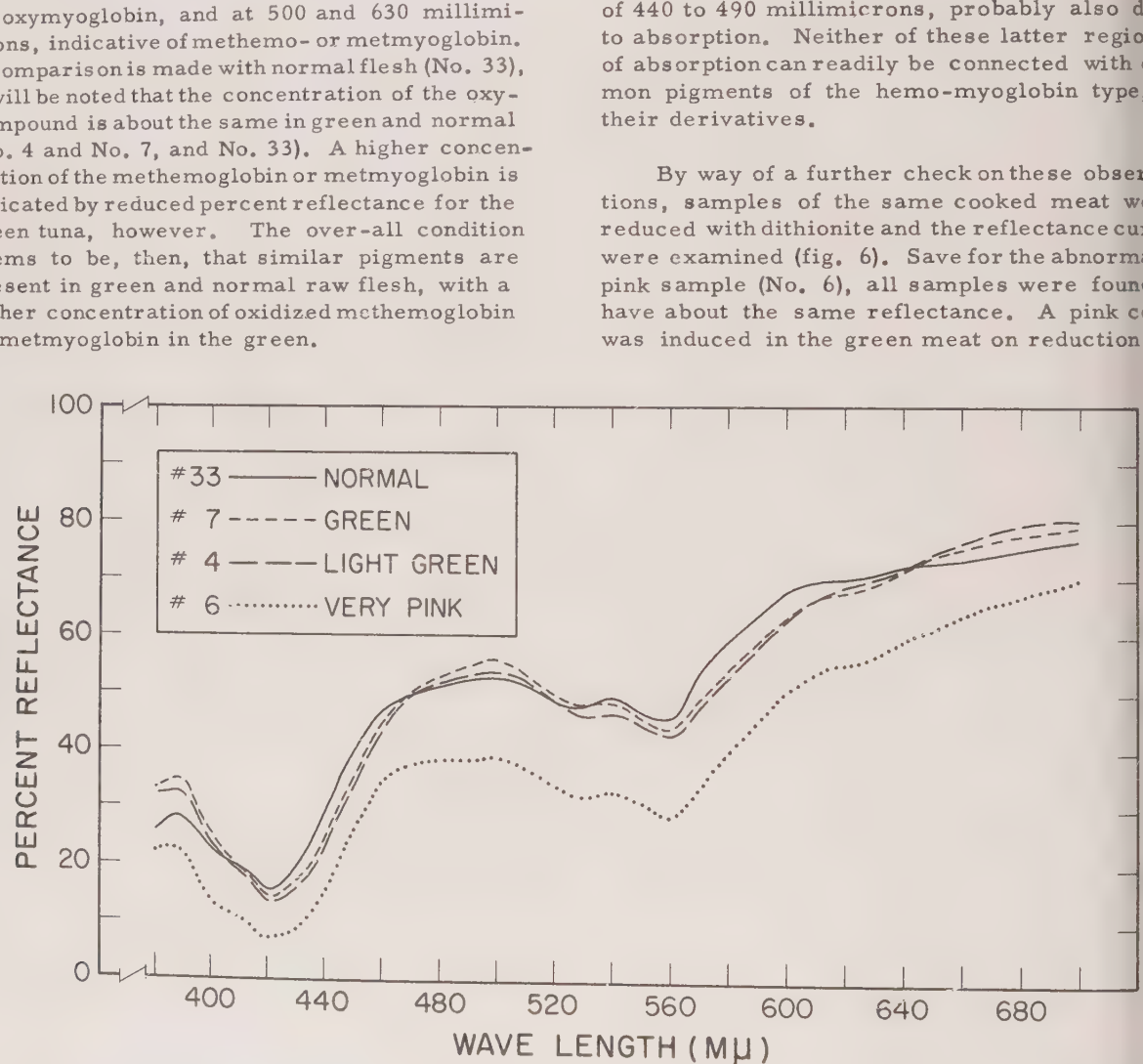

Figure 6.--Reflectance of reduced cooked tuna of various colors in the unreduced state.

Table 1.--Types and relative heme pigment content in raw and cooked tuna. Basic heme pigment is assumed to be myoglobin

	Oxy-myoglobin	Met-myoglobin	Denatured globin	
			hemichrome	hemochrome
	Raw Tuna		Cooked Tuna	
Normal	++	+	+	++
Green	++	++	++	++

\+ = low concentration

++ = higher concentration

icant slight absorption of unknown source
oted at about 480 millimicrons for normal
hat was present to but a slight extent, or
t, in the curves for green meat. The prom-
absorption maxima at 530 and 560 millimi-
are the peaks characteristic of denatured
in hemochrome, the reduced form of the
hrome pigment noted in the other cooked
es. The results of these studies on the
tance curves for green and normal, and
gments that are indicated as being present,
mmarized in table 1.

was noted that a copious quantity of yellow-
d leach liquor was obtained during the pro-
f cooking tuna flesh in the laboratory. Also
found that a deeper pigmentation was pres-
the leachate from the normal tuna than that
the green, indicating some correlation.
ached material was susceptible to oxida-
ith oxygen and reduction with dithionite,
ve no characterizing absorption curve.
ts to purify by column chromatography
nconclusive. Laboratory cooking of nor-
nd green tuna flesh in contact with leach
form normal or green tuna had no effect
color of the samples.

CONCLUSIONS

om the above investigations we have
d the tentative conclusions that (1) the
hanges, including greening, that take place
flesh on cooking are the result of reactions
normal pigments of the flesh (hemo- or
bin or their derivatives) with other
ients of the flesh or with the environment,
) at this stage of the work we are inclined
belief that differences between normal and
flesh lie chiefly in the differences in con-
tion of certain of the common heme pig-
derivatives. Some evidence also exists for
concentration of additional pigments in
green meat, which may be unusual hemi-
globin derivatives. Browning and greening
o be manifestations of oxidation of the heme protein pigments. How these pigment factors are related to the physiological or physical condition of the fish flesh remains to be investigated.

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NOTES

Tagged Bigeye Tuna Recovered

A bigeye tuna *(Parathunnus sibi)* tagged by the Pacific Oceanic Fishery Investigations in the North Pacific was recently recaptured by the Japanese during their winter longline operations in waters north of the Hawaiian Islands, marking the first known bigeye tag recovery in the Pacific.

The fish was tagged on January 31, 1955, by the U. S. Fish and Wildlife Service research vessel *John R. Manning* (cruise 23), at 30° 59′N, 171°14′W, which is about 360 miles northeast of Midway Island, during an albacore survey cruise to the North Pacific. The tuna, captured by longline, measured 122.3 cm. in fork length at the time of release.

The recovery was made by a Japanese longline vessel, the *No. 5 Nikko Maru,* of the Nikko Fishing Company of Fukushima Prefecture, on November 24, 1955, at 32°41′N, 155°57′W, approximately 700 miles due north of Oahu, T. H. The straight-line distance between the points of release and recovery is 800 miles. The length of the fish at recapture was 126.8 cm., indicating a net gain in length of 4.5 cm. during the interval of 10 months. In terms of estimated weight this represents a gain of approximately 10 pounds.

This recovery was the first from a total of 82 bigeye tuna tagged and released in North Pacific waters by POFI between January, 1954, and September, 1955. The tag, in excellent condition, together with pertinent information regarding its recovery, was returned to the Pacific Oceanic Fishery Investigations laboratory through the cooperation of Mr. Shoji Ueyanagi of the Nankai Regional Fisheries Research Laboratory of Kochi, Japan. The tag used was a type "G" vinyl plastic tube tag recently developed by the California Department of Fish and Game (Wilson, R. C., 1953, Calif. Fish and Game 39(4):429-442).—*Tamio Otsu and Richard N. Uchida, Pacific Oceanic Fishery Investigations, U. S. Fish and Wildlife Service, Honolulu.*

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